Spatial and Seasonal Variability of Earthworms in Wheat Fields of the Inland Pacific Northwest

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Authorization to Submit Thesis

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Abstract

Spatial and temporal variations in soil conditions are expected to constrain earthworm-mediated benefits to crops and soil health in the Inland Pacific Northwest (IPNW). The objectives of this work were to describe the distribution and diversity of IPNW earthworms across regional and seasonal variation in soil conditions. In the springs of 2012-2013, 36 sites across the IPNW were sampled for earthworms and their density and diversity were measured. *Aporrectodea trapezoides* was the dominant species at all sites and a threshold for earthworm presence was observed at 330-370mm mean annual precipitation. A second study measured earthworm density and activity over 14 months at six sites in the annual cropping zone of the IPNW. Mean densities of 9 to 149 individuals m^{-2} over a 121-day active period were recorded. This work provides a starting point for determining the effects of earthworms on regional crop production and the potential impacts of climate change.

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Chapter 1: Background

Earthworms in Agriculture

The ability of the earthworm to transform its environment, altering soil structure and chemical properties (Baker et al., 2006; Carpenter et al., 2008; Costello and Lamberti, 2009; Coulis et al., 2014; Darwin, 1862; Jones et al., 2006; Lavelle et al., 1997; Syers and Springett, 1984) has earned this unassuming organism the title of "ecosystem engineer" (Blouin et al., 2013). In agricultural ecosystems, earthworms have been credited with increasing crop yield an average of 25-35% (Brown et al., 1999; van Groenigen et al., 2014). Enhanced nitrogen mineralization is thought to account for most of this increase in yield (Blair et al., 1997; van Groenigen et al., 2014).

Earthworms consume residue from the soil surface or organic matter in the subsurface soil. Alterations during passage through the earthworm gut result in significant changes in microbial community structure, carbon to nitrogen ratios, and nutrient availability (Eisenhauer et al., 2007). The effects on nutrient availability in particular are thought to be an indirect result of changes to the microbial community (Eisenhauer et al., 2007). Microbial abundance in earthworm guts, casts and burrows surfaces is generally greater than in the bulk soil (Drake and Horn, 2006; Drake and Horn, 2007; McLean et al., 2006).

Earthworm effects on soil properties, and thereby on crop growth, are not universal but depend on earthworm species, density, and environmental variation (Blouin et al., 2013). Results of one meta-analysis suggest that crop production in tropical systems may be increased up to 40% when earthworm biomass is at least 30 g per m^2 (Brown et al., 1999). Another meta-analysis reports differences in earthworm effects based on earthworm density, soil type, fertilizer type, amount of crop residue, soil disturbance and soil pH (van Groenigen et al., 2014). Earthworm effects were greater for basic soils and for clayey soils compared to sandy or loamy (van Groenigen et al., 2014). Systems dependent on organic sources of nitrogen or receiving more than 6000 kg ha^{-1} yr^{-1} of organic residues also benefited significantly more from earthworm presence (van Groenigen et al., 2014). Earthworms had significant effects at all densities but these effects were significantly greater at earthworm densities greater than 400 individuals per m^2, compared to <100 per m^2 (van Groenigen et al., 2014). Combined these effects suggest that earthworms may be most important in systems where producers are transitioning from inorganic to organic nutrient sources.

IPNW Wheat Production

The Inland Pacific Northwest (IPNW) region is a major wheat producing region, spanning eastern Washington, northeastern Oregon and northern Idaho (Schillinger and Papendick, 2008). This region produces more than 130 million bushels of wheat annually (more than $500 million annually) (Mackun, 2009). Approximately 85% of the wheat produced within the IPNW is grown under dryland (un-irrigated) conditions (Mackun, 2009). Located between the Cascade and Rocky Mountain ranges this region is characterized by a Mediterranean climate, with cool wet winters, and hot dry summers (Papendick, 1996).

A marked precipitation gradient exists with mean annual precipitation varying from 250 mm in the west to over 500 mm in the east with 75% of precipitation occurring in the winter (Papendick, 1996). The soils of the region are primarily Mollisols and Aridisols with some Alfisols in the higher rainfall regions (Rasmussen et al., 1998). On the eastern edge of the greater IPNW lies the Palouse region (Black et al., 1998; Schillinger and Papendick, 2008). This region is known for its deep loess soils and rolling hills, (Schillinger and Papendick, 2008). Whitman County, Washington, occupying a large portion of the Palouse region, consistently has the highest dryland wheat yields in the country (WGC, 2014). In response to the steep precipitation gradient and variation in soils, agronomic practices and crop species vary throughout the region (Schillinger and Papendick, 2008).

In this dryland region, the availability of water is a driving factor in determining management practices and productivity and is dependent on precipitation, as well as other properties such as topography, and soil characteristics (Douglas et al., 1992; Papendick, 1996). The range of agronomic practices in the IPNW can be generalized by four agroecological classes (AECs) based on the dominant cropping system at any given location. The four AECs used in this work are annual cropping (a crop in every year), transition cropping (e.g. rotations with fallow in one year in three or less), crop-fallow (2 year rotation of crop and fallow) and irrigated (Kaur et al., 2014).

Earthworms in the IPNW

The distribution of native earthworms in North America is primarily a product of the Wisconsinan Glaciation, which ended 12,000 years ago, combined with the impacts of European colonization beginning around 400 years ago (Hendrix, 1995). First the Wisconsinan Glaciation extirpated any native earthworm species from soils affected by ice sheets and permafrost. Subsequently, a variety of disturbances associated with the activity of European settlers have further reduced the extent of native species and, perhaps more importantly, have resulted in the spread of numerous invasive species, primarily from Europe and Asia (Callaham et al., 2006; Hendrix and Bohlen, 2002; Hendrix et al., 2008). Known pockets of remaining native species are concentrated in the relatively warm and humid Pacific Northwest, southeastern US and parts of southern California and Mexico (Callaham et al., 2006; Hendrix and Bohlen, 2002; Hendrix et al., 2008). Fender (1985) lists five native genera in the Columbia basin area *Driloleirus, Drilochaera, Argilophilus, Arctiostrotus,* and *Macnabodrillus*. Within these 5 genera, only 3 species with limited distribution have been described. For example, *Drilochaera chenowithensis* has been found only at one site along the Columbia River west of The Dalles, Oregon. *Argilophilus hammondi* was found at this same site and a site in the Ochoco National forest in Crook County, Oregon (Fender, 1985). *Driloleirus americanus* is the only known native earthworm in the Palouse region and had been thought to be extinct since 1978 (James, 2000). Extensive cultivation and conversion of native grassland to small grain annual cropping (Black et al., 1998) greatly reduced habitat for this species (Sanchez-de Leon and Johnson-Maynard, 2009). It is only recently that specimens of *D. americanus* have been rediscovered in native Palouse Prairie remnants (Sanchez-de Leon and Johnson-Maynard, 2009).

In the 400 years following European colonization of the continent exotic earthworms have spread and currently inhabit all but the driest and coldest habitats in North America (Hendrix et al., 2008). An estimated 120 exotic earthworm species can now be found in the United States and Canada (Hendrix et al., 2008). In the IPNW earthworm communities are dominated by exotic species including *Aporrectodea chlorotica, Aporrectodea rosea, Aporrectodea trapezoides, Aporrectodea tuberculata, Aporrectodea turgida, Dendrodrilus rubidus, Eisenia tetraedra, Eisenia fetida, Lumbricus terrestris, Lumbricus rubellus, Octolasion cyaneum,* and *Octolasium tytraeum* (Fauci and Bezdicek, 2002; James, 2000; Sanchez-de Leon and Johnson-Maynard, 2009; Umiker et al., 2009). The lowest diversity of earthworm species in the region has been found in agricultural fields (Fauci and Bezdicek, 2002). In a 2002 survey of earthworm species, *A. trapezoides* was the most common species in agricultural fields, and *Aporrectodea tuberculata*, *Lumbricus terrestris,* and *Aporrectodea* *turgida* were the only other species found in these fields (Fauci and Bezdicek, 2002). While previous studies have provided a baseline for earthworm species present in this region (Fauci and Bezdicek, 2002; James, 2000; Sanchez-de Leon and Johnson-Maynard, 2009; Umiker et al., 2009; Xu et al., 2013), there has been no systematic survey to determine earthworm population density or biomass in this region, or to connect variations in earthworm populations to climatic or other environmental variables.

Ecological Groups of Earthworms

One way the behavior of earthworms has traditionally been generalized is through the use of three ecological groups, anecic, epigeic and endogeic (Bouché, 1977). However, many species do not fit neatly into one of these categories leading to the proposal of intermediate categories such as endo-anecic and endo-epigeic (Brown, 1995; Lavelle et al., 1998). Endogeic species generally create horizontal, non-permanent burrows with in the upper layers of the soil profile, consuming organic matter within the soil (Bouché, 1977). Within the endogeic group there are subcategories based on the soil layer they inhabit. Species that feed on the organic matter rich topsoil layers, such as *A. trapezoides* are referred to as polyhumic species and are generally smaller in size than the mesohumic and oligohumic species, which inhabit deeper soil layers. Oligohumic species inhabit the mineral soil and are larger in size while mesohumic species inhabit the A horizon and fall between the other two categories in size. *Aporrectodea trapezoides* is usually classified as a polyhumic endogeic species. Epigeic earthworms are active at the interface between the organic litter layer and the soil surface, do not burrow in the soil, and are rare in agroecosystems (Lee, 1985; Shipitalo and Le Bayon, 2004). Anecic species create permanent vertical burrows reaching deep into the soil profile and pull organic matter from the soil surface into their burrows to be consumed (Lee, 1985). Anecic species are favored in systems with minimal soil disturbance and can incorporate large amounts of surface residue into the soil (Edwards and Lofty, 1980). Anecic species seem to be more rare in cropping systems than endogeic species (Fragoso et al., 1997; Kladivko et al., 1997); though there is some evidence that anecic species may move back into fields under long-term, no-till management (Kladivko et al., 1997).

Environment, Climate and Earthworm Distribution

As soft-bodied organisms earthworms are highly sensitive to environmental conditions. Earthworms respond to an interaction between soil temperature and soil moisture and earthworms are generally able to withstand greater temperature extremes with adequate moisture (Eriksen-Hamel and Whalen, 2006; Perreault and Whalen, 2006; Presley et al., 1996; Richardson et al., 2009; Wever et al., 2001). One of the primary ways soil moisture and temperature can affect earthworm activity is by inducing aestivation. Aestivation is a form of diapause in which the earthworm forms a spherical aestivation chamber in which it remains in a tightly coiled, dormant state until soil conditions are once again favorable (Juan et al., 2000; Lee, 1985).

In laboratory experiments, thresholds for aestivation vary with soil temperature and can occur at water contents as high as 25% (Wever et al., 2001). Water potential thresholds from laboratory experiments range from -2 to -20 kPa (Hindell et al., 1994; Holmstrup, 2001). While earthworms are negatively affected by low soil moisture, they are also sensitive, to a lesser degree, to saturated soil conditions and may avoid these conditions (Roots, 1955). However, earthworms can survive immersion in aerated water for weeks (Roots, 1955).

While soil moisture is generally the most important factor driving earthworm survival, differences in soil temperature are also important in determining earthworm growth rates and mortality (Baker and Whitby, 2003; Richardson et al., 2009; Wever et al., 2001). For *A. tuberculata*, a close relative of *A. trapezoides,* soil temperatures greater than 25° C were fatal when soil moisture ranged from 5 to 25%, increasing temperature up to 20° C reduced survival at all moisture levels below 25% (Wever et al., 2001). Without a better understanding of *A. trapezoides* sensitivity to the soil moisture and temperature regime of the INPW it is difficult to predict the active period, and by extension, the potential impact of the species in this region.

Conclusion

Earthworms have earned their title as ecosystem engineers through their ability to have dramatic effects on ecosystems through extensive modification of soil physical and chemical properties (Blouin et al., 2013). In agricultural ecosystems, their activity may play an important role in mineralizing organic matter to plant available nutrients, particularly agroecosystems dependent on organic nutrient sources (van Groenigen et al., 2014). Positive earthworm effects on crop production have been observed across a wide range of climates, soil types, and cropping systems but the magnitude of these effects is dependent on the density and species of earthworms present, among other factors (van Groenigen et al., 2014). Surveys have begun to document the diversity of earthworm species in this region but there has been no comprehensive survey attempting to document the distribution and density of earthworms across the range of climates in the IPNW. Finally, it is not known how seasonal variation in IPNW soil conditions effects the active period of earthworms in this region, which is in turn expected to limit earthworm effects on soil properties.

This thesis research will explore the role of climate and other environmental factors in determining the spatial and temporal distribution of earthworms in the IPNW. The specific objectives of this research include:

1. Determining the spatial distribution of earthworms across the IPNW.
2. Assessing the role of climate and environmental factors in determining this distribution.
3. Determining the earthworm species present in agricultural fields of this region.
4. Describing the temporal variability in earthworm densities in Palouse agricultural fields.

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Chapter 2: Earthworm Distribution and Density across a Climatic Gradient within the Inland Pacific Northwest Cereal Production Region

Submitted to *Applied Soil Ecology*

Abstract

Despite their recognized importance in promoting crop yields and soil health, little is known about earthworm species composition and density within the cereal production region of the Inland Pacific Northwest (IPNW). The overall goal of this work was to determine the distribution earthworm species and the density of earthworm populations in the region. Sites were selected across a climatic gradient to represent three agroecological classes (AECs), annual cropping, transition crop-fallow, and crop-fallow. Earthworm density was measured at each site and adults were collected and identified to species in 2011, 2012 and 2013. Soil samples were taken from each site to determine soil pH, texture and organic matter but no relationship with earthworm distribution was observed for these measures. Earthworm diversity was extremely low with only a single species, *Aporrectodea trapezoides,* found at most sites. *Aporrectodea trapezoides* was present at lower mean annual precipitation (MAP) than previously reported, 370 mm, but was not found at any sites with lower MAP. This may indicate a climatic limit on the distribution of this species in the region. The only other species collected, *Lumbricus terrestris,* was found only at two annual cropping sites. Earthworms were present at all sites within the annual cropping and transition classes, while only 13.3% of sites within the crop-fallow AEC had earthworms. The mean earthworm biomass at sites with earthworms, 94.3 g m^{-2} was above those reported to be necessary to significantly influence crop yields in other systems, suggesting that further research focusing on quantification of the impact of earthworm on crop yields in this important agricultural region is warranted.

Introduction

Earthworms are widely recognized as ecosystem engineers (Blouin et al., 2013). This status is largely tied to the significant impact earthworm activity has on soil structure and chemical properties (Baker et al., 2006; Carpenter et al., 2008; Costello and Lamberti, 2009; Coulis et al., 2014; Jones et al., 2006; Lavelle et al., 1997; Syers and Springett, 1984). In agroecosystems, earthworms have positive impacts on plant production with average increases in yield of 25-35% reported in meta-analyses (Brown et al., 1999; van Groenigen et al., 2014) and in tropical ecosystems an earthworm biomass of 30 g m^{-2} are required to increase crop yield by 40% (Brown et al., 1999). The primary mechanism for this increase is thought to be via changes in nitrogen availability (Blair et al., 1997; van Groenigen et al., 2014). Significant earthworm effects may be expected at all densities but the magnitude of this effect is dependent on earthworm density, soil type, fertilizer type, amount of crop residue, soil disturbance and soil pH (van Groenigen et al., 2014), as well as species composition (Blouin et al., 2013). The potential positive impacts of earthworms on crop yields and the species/density-dependent nature of these interactions, suggest the importance of quantifying species density and composition in agroecosystems. While the impact of earthworms on crop yields when large quantities of chemical fertilizers are applied is likely to be minimal, earthworms could play an important role in systems transitioning to organic fertilizers (van Groenigen et al., 2014).

Many factors are known to control earthworm density and the presence of specific earthworm species. The importance of each factor varies, dependent on the scale at which earthworm distribution is being addressed. Spatial variation in earthworm distribution (presence/absence) at the coarse, regional scale is primarily related to measures of environmental suitability. Climatic factors such as mean annual precipitation (MAP), winter low temperatures and summer high temperatures drive distribution based on species-specific tolerances to moisture and temperature extremes (Curry and Schmidt, 2007; Holmstrup and Overgaard, 2007; Holmstrup and Zachariassen, 1996; Meshcheryakova and Berman, 2014; Nieminen et al., 2011; Richardson et al., 2009; Thonon and Klok, 2007). Because earthworms spend most of their time within the soil or protected by litter at the soil surface, the effects of climatic measures like air temperature and MAP do not directly impact earthworms. Rather, these measures are modified by factors such as soil depth, texture and drainage into more earthworm-relevant measures such as soil moisture and soil temperature (Curry and Schmidt, 2007; James, 2000; Nieminen et al., 2011). Soil texture is thought to primarily effect earthworms by determining water holding capacity, with earthworms generally avoiding coarse textured soils that readily drain (Lee, 1985) or heavy clay soils, which may be prone to anaerobic conditions (Curry and Schmidt, 2007).

Within regions with suitable climates and soil conditions for earthworms there can still be significant variation in species composition and population density as a result of local heterogeneity of soil factors. At the field and landscape scale, properties such as soil moisture and temperature (Cannavacciuolo et al., 1998; Johnston et al., 2014; McCredie et al., 1992; Millican and Lutterschimdt, 2007; Snyder et al., 2010), soil texture (Millican and Lutterschimdt, 2007), food availability (Johnston et al., 2014), soil pH (Curry and Schmidt, 2007) and management (Ernst and Emmerling, 2009; Kladivko et al., 1997; Nieminen et al., 2011; Pelosi et al., 2013) influence earthworm density and contribute to the natural tendency of earthworm populations to have spatially patchy distribution (Gutierrez-Lopez et al., 2010; Jiménez et al., 2001; Valckx et al., 2009; Whalen, 2004). Earthworms respond to local variations in pH and generally avoid pH values of less than 4.5, preferring values between 5.0 and 7.4 (Curry and Schmidt, 2007). Soil organic matter primarily influences earthworm distribution by determining quantity and quality of food (Johnston et al., 2014; Ouellet et al., 2008).

Temperatures and moisture values above or below tolerance thresholds of earthworm species result in mortality or induce responses to avoid or wait out unfavorable conditions (Curry and Schmidt, 2007; Lee, 1985). These responses include aestivation and migrating to lower, cooler portions of soil (Curry and Schmidt, 2007; Lee, 1985). Lack of activity or deep burrowing may negate or limit the positive influence of earthworms on crop growth and yield. Since the ability of earthworms to survive warm, dry periods depends on species and ecological niches (Eggleton et al., 2009; Millican and Lutterschimdt, 2007) it is critical to understand the composition of earthworm populations in addition to population densities in managed ecosystems.

The Inland Pacific Northwest (IPNW) is an important cereal production region that spans southeastern WA, western ID and northeastern OR (Fig. 1). The IPNW is characterized by a Mediterranean-type climate, featuring cold, wet winters and warm to hot, dry summers (Papendick, 1996). A marked precipitation gradient exists with mean annual precipitation varying from 25 cm in the west to over 50 cm in the east with 75% of precipitation occurring in the winter (Papendick, 1996). The soils of the region are primarily Mollisols and Aridisols with some Alfisols in the higher rainfall regions (Rasmussen et al., 1998). Agricultural production in the IPNW is generally dryland, with irrigated lands in the drier, western edge. The sampling region is categorized by four agroecological classes (AECs) based on the dominant cropping systems at any given location. Designations were based on the National

Agricultural Statistics Service CropLand data layer (USDA, 2012). The four AECs are annual cropping (limited annual fallow), transition cropping (e.g. rotations with fallow, but not in every year), crop-fallow and irrigated (Kaur et al., 2014). Many factors contribute to a producer's ability to grow a crop annually (Douglas et al., 1992) and farmers may be able to produce a crop more or less frequently than climate alone would predict. Based on actual ratios of crop to fallow, this agroecological classification system is able to incorporate the many factors that contribute to a farmer's management decisions and dictate the actual limits imposed on crop production. In this dryland region, soil water is the primary limiting factor on crop production therefore these management based classifications provide insight into the moisture holding capacity and water availability of soils, factors that are also very important to moisture sensitive earthworms.

Little is known regarding how earthworm populations change across this productive agricultural region in relation to climate. Previous earthworm surveys summarized by James (2000) reported a total of 11 exotic species and 5 native genera (3 described species) in the Pacific Northwest but did not report densities. These surveys covered a wide range of habitats and while some agricultural sites were included they were not the focus of these surveys. Fauci and Bezdicek (2002) conducted a survey of 46 sites within the higher rainfall areas of the IPNW known as the Palouse in eastern Washington and northern Idaho, focusing primarily on agricultural fields (n=29) and adjoining perennial vegetation, old home sites and waterways (n=17). Like the earlier surveys, Fauci and Bezdicek (2002) only reported species composition and not density. A total of 10 lumbricid species were identified, with the greatest diversity recorded in waterways and low lying areas (Fauci and Bezdicek, 2002). Agricultural fields had the lowest diversity, with 11 fields having no earthworms, 11 only having one species, *Aporrectodea trapezoides* (Dugés), and only 7 sites having two or more species (Fauci and Bezdicek, 2002). *Aporrectodea trapezoides* was the most common species in agricultural fields followed by *Aporrectodea tuberculata* (Eisen), *Lumbricus terrestris* (L.), and *Aporrectodea turgida* (Eisen); no other species were found in agricultural fields (Fauci and Bezdicek, 2002). Published estimates of springtime earthworm density that are available for cereal production systems in the annual cropping class range between 20 and 110 earthworms m^{-2} (Johnson-Maynard et al., 2007; Umiker et al., 2009). Similar to the Fauci and Bezdicek (2002) survey, these studies also found *A. trapezoides* to be the dominant species present.

Despite the importance of soil biota in general and earthworms in particular, little is known about the interactions between above and below ground biota and exactly how these interactions will be impacted by global change (Hooper et al., 2000; Wolters et al., 2000). The role of earthworms as ecosystem engineers, their interactions with other above and below ground organisms, and their ability to influence agricultural efficiency and crop production point to earthworms' important role in agriculture's response to a changing climate. However, before we can predict the effects of climate change on earthworm populations, we first need a better understanding of the current distribution and densities of earthworms in agricultural systems. We also need a better understanding of the role of climate and soil physical properties in driving spatial distributions and the active period of earthworms. The objectives of this work, therefore, are to identify earthworm species composition and population densities in agricultural fields of the Inland Pacific Northwest (IPNW), an important cereal production region in the U.S., and identify soil and climatic factors important in determining earthworm distribution and density.

Materials and Methods

Field Sampling

Individual fields (n=36) were selected from the annual cropping, transition, and crop-fallow AECs (Fig. 2.1). All fields were planted to winter wheat *(Triticum aestivum)* at the beginning of the study. Different pits in the same fields were sampled in each year of the study. Due to crop rotation, the crop type changed from year to year. Winter wheat and spring wheat were the most common crops with barley, canola, safflower, garbanzo beans or lentils as rotational crops at some sites. Approximately two thirds of sites utilized conservation tillage in at least part of their rotation. The irrigated zone was not sampled because of limited field site availability and the confounding impact of added soil moisture on earthworm survival. Initial sampling in 2011 included 19 sites. Sampling in 2012 and 2013 included a total of 36 sites (including 11 in annual, 10 in transition and 15 in crop-fallow). Sampling was conducted during May and June in each year when soil moisture levels were relatively high. Earthworms were sampled using a combined hand sorting and sieving technique. Earthworms were collected from two 25x25x50 cm pits at each site. Sites were sampled generally moving from west to east to allow sampling during a period of high soil moisture for each site when earthworm activity was expected to be similarly high. During sampling volumetric soil moisture values ranged from 11% to 44% across all sites in 2012 and 2013 (soil moisture data was not collected in 2011), the minimum soil moisture at sampling for sites with worms was 13%. Soil temperature ranged from 10 to 30°C within the top 30 cm of soil. In 2012, soil samples were collected from one pit at each site in 10 cm depth intervals at the time of earthworm sampling.

Laboratory Analyses

Earthworms and soil samples were transported to the laboratory in a cooler. Once in the laboratory, live earthworm biomass was obtained after incubating earthworms at 15°C for 48 hours on filter paper saturated with a 1:8 Ringer's solution. Adult earthworms were euthanized and preserved in formalin. Adult specimens were identified to species using Schwert's key to Lumbricidae (Schwert, 1990).

Particle size distribution for each site was measured using the hydrometer method (NRCS, 1992). Soil pH was measured using a 1:1 (soil:water) dilution (NRCS, 1992) and total organic matter was measured using the loss on ignition method (NRCS, 1992). Also, modeled annual and seasonal precipitation and temperature data were obtained from the West Wide Drought Tracker Service. Temperature and precipitation data are based on climatologically-aided interpolation, from the Oregon State University PRISM Climate group (PRISM, 2014). This data set uses all available station networks and data groups to produce stable, fine-scale spatial models of precipitation and temperature (PRISM, 2014). Mean annual precipitation and mean annual temperature (MAT) are based on 30-year averages for 1981-2010. Spring precipitation (March-June) and winter precipitation (September-February) are based on a 3-year average for 2011-2013.

Statistical analyses were carried out in SAS version 9.3 (SAS, 2011). Spearman rank-order correlations were run using three-year means for biomass and density for sites where earthworms were present. The data were analyzed as a completely randomized design with repeated measures (years). Analysis of variance used a generalized linear model assuming a negative binomial for density and a log norm for biomass in addition to an autoregressive correlation structure between years (Stroup, 2014). Year and AEC effects on earthworm density and biomass were analyzed for only those sites with earthworms present. Effect of AEC on site characteristics (pH, soil organic matter, MAP, MAT, spring precipitation, winter precipitation, soil texture) was analyzed using all sites using a general linear model analysis of variance.

Results and Discussion

Agroecological Classes

Regional scale distributions of earthworms are determined by measures of environmental suitability including soil factors such as pH and texture (James, 2000; Nieminen et al., 2011) and climatic factors such as precipitation and temperature which determine seasonal soil moisture and temperature extremes (Holmstrup and Overgaard, 2007; Holmstrup and Zachariassen, 1996; Meshcheryakova and Berman, 2014; Nieminen et al., 2011; Richardson et al., 2009; Thonon and Klok, 2007). Both seasonal and annual measures of precipitation are the only climatic variables that differed significantly among AECs, with no differences among AECs for MAT (Table 2.1). As expected, all measures of precipitation were highest in annual crop fields (mean MAP 565 mm) where rainfall is sufficient to allow a crop in every year and lowest in the crop-fallow fields (mean MAP 313 mm) where crop-fallow rotations allow rainfall to be stored in the soil in alternate years. Despite the broad range in precipitation among sites (220-645mm MAP across all sites) there were no significant differences among AECs for soil pH, texture and organic matter; suggesting that these factors are unlikely to play an important role in regional earthworm distribution. Soil pH in the top 30 cm in the region ranged from 4.8 to 7.7 and earthworms were found across this range of (Table 2.2). Soil textures in the top 30 cm varied little (Table 2.2) with textural classes ranging from sandy loam to clay loam. Earthworms were found in sites with sand size fractions as high as 54% and clay fractions as great as 30% across the sites. Soil organic matter at sites with earthworms varied greatly, from 1.6 to 8.3%.

Earthworm Diversity, Density and Biomass

Earthworms were found in 21 of the 36 sites surveyed in 2012 and 2013 and 12 of the 19 sites surveyed in 2011. Over the three years of the study, earthworms were present at all of the annual and transition fields and only 13.3% of crop fallow fields (Table 2.1). For all sites with earthworms present, earthworms were found in every year. Fields with no earthworms were earthworm free in every year of the study.

Aporrectodea trapezoides was the only earthworm species identified at all but two sites. A single adult *Lumbricus terrestris* was found at two sites within the annual cropping class in a single year only. The hand-sorting technique employed in this survey likely under represents anecic species such as *L. terrestris* whose burrows extend below the sampling depth (Callaham Jr and Hendrix, 1997; Lawrence and Bowers, 2002). It is possible, therefore, that anecic species are underrepresented in this report, especially in the conservation tillage fields in the high rainfall zone. Anecic species form permanent vertical burrows, feeding on organic matter from the surface and drawing it deep into the soil profile (Bouché, 1977; Lee, 1985). These species tend to be favored in lower disturbance, conservation tillage systems, while endogeic species (those that form non-permanent burrows and feed on soil organic matter) such as *A. trapezoides* tend to dominate in higher disturbance systems (Kladivko, 2001; Kladivko et al., 1997; Nuutinen, 1992). In 2002, Fauci and Bezdicek (2002) also reported *A. trapezoides* as the dominant species in agricultural fields but reported a total of four species and found two or more species at 7 of 18 sites with earthworms present. The lower diversity in our study may be explained by a combination of factors. First, Fauci and Bezdicek's 2002 survey sampled 10 or more points within each field in order to capture the highest diversity of species. The necessity of sampling across a large geographic area as soil moisture declined limited sampling to two pits per field, which would have reduced the chances of detecting less abundant species. Earthworm specie's tendency to distribute patchily necessitates a high density of pits per m^2 to capture variation across a landscape (Whalen, 2004). Second, in the 12 years since the 2002 survey, distributions and abundances of some or all of species may have shifted.

Earthworm biomass across the region varied between 0.4 to 849 g m^{-2} (mean 89, median 38 g m^{-2}) and density from 2 to 458 individuals m^{-2} (mean 97, median 71 m^{-2}). The upper end of these values are much greater than those previously reported in the region (Johnson-Maynard et al., 2007; Umiker et al., 2009) and well over reported thresholds for significant impacts on crop production (Brown et al., 1999; van Groenigen et al., 2014). However, 17 of the sites with earthworms had biomass below 30 g m^{-2} in at least one sampling year. There were no significant differences among AECs for earthworm biomass or density (Fig. 2.2). Year effects were significant for biomass ($p<0.0001$) and density ($p<0.01$). This year effect reflects unusually high values for density (227.1 individuals m^{-2}) and biomass (mean=312g m^{-2}) in 2011 (Fig. 2.3). In 2012 and 2013, mean earthworm density (105.0 individuals m^{-2}) and biomass (64.5 g m^{-2}) were similar to previous estimates for the region, which estimate earthworm density as 62.5 individuals m^{-2} and biomass as 36.6g m^{-2}. Differences in annual precipitation may have contributed to the high earthworm populations in 2011. Mean precipitation for all sites was 276 mm in winter before 2011, but only 181 mm prior to 2012 and 226 mm prior to 2013.

Earthworms and Climatic Variables

Aporrectodea trapezoides, the dominant earthworm collected at all of our sites, is widespread across the U.S. and is referred to as an exotic-invasive species (Fernandez et al., 2011; Sanchez-de Leon and Johnson-Maynard, 2009). The success of this earthworm is attributed to factors such as rapid reproduction, parthenogenesis and adaptability (Fernandez et al., 2011; Fernández et al., 2010). The region sampled in this study experiences dry, warm summers that may restrict some earthworm species. *Aporrectodea trapezoides* are able to survive these conditions by aestivating (Lee, 1985; McDaniel et al., 2013). In this form *A. trapezoides* is able to remain dormant, conserving moisture, until soil conditions are again favorable. However, there appear to be limits to this ability. Previous work has suggested a threshold of 600 mm MAP for significant decreases in *A. trapezoides* density in cropping fields and pastures of southwestern Australia where the cool and wet seasons also coincide but soil temperatures are generally higher (Mele and Carter, 1999). In the Australian study earthworms were present at all sites and the lowest MAP site sampled received roughly 330mm (Mele and Carter, 1999). In crop fields of the IPNW, the lowest MAP site received 220 mm and a threshold between 330 and 370 mm MAP was observed below which earthworms were not detected (Fig. 2.4). All sites without earthworms fell below 330 mm MAP, while the lowest MAP for sites with earthworms was 370 mm. This threshold may mark the point at which inhospitable conditions persist long enough to exhaust reserves of dormant, aestivating *A. trapezoides* or the corresponding hospitable period may be too short to allow populations to build to stable numbers, or both. The one exception to the pattern observed in this study is an experimental site at a research station in Pendleton, OR, which had MAP of 436 mm but no earthworms. These samples were taken from with in a long-term tillage and while earthworms were not found in a conventional tillage treatment, they were present (mean biomass 310 g m^{-2}) in the no-till treatment. In environments with marginal climatic suitability, management may play a greater role in determining overall environmental suitability. To our knowledge, the MAP values in our study are among the lowest that have been reported for *A. trapezoides*. Reported MAP for locations with *A. trapezoides* (Crumsey et al., 2014; Garnsey, 1994; McCredie et al., 1992) are similar to or higher than the higher rainfall sites included in this study.

Although a MAP threshold performs well for predicting presence/absence of *A. trapezoides*, and the patterns appear to be consistent with water stress related limitations for this species, the relationship with MAP and earthworm density is not consistent with this interpretation. While earthworm density has previously been found to positively correlate with MAP (Mele and Carter, 1999), in this study density had a significant negative correlation with fall (coefficient=-0.6792, $p<0.01$) and spring (coefficient=-0.6091, $p<0.01$) precipitation (Table 3). Earthworm biomass was also significantly negatively correlated with the previous winter's precipitation (coefficient=-0.7377, $p<0.001$), previous spring's precipitation (coefficient=-0.6779, $p<0.01$) and MAP (coefficient=-0.4390, $p<0.05$) (Table 2.3). These negative correlations are observed when looking only within sites where earthworms were present. Earthworms were present in all annual and transition fields and absent from 86.7% of crop-fallow fields. Mean earthworm biomass in crop-fallow fields decreased from 202.5 to 29.8 g m^{-2} and from 151.2 to 18.6 individuals m^{-2} when sites without earthworms are included. The data suggest that while earthworms are not common in fields within the drier, crop-fallow AEC, stable and relatively high densities may be found when conditions are suitable.

Unlike the steep gradient in MAP across this region there is little variation in MAT (46.4 to 51.8°C). This range in MAT is likely too narrow to act as a driving factor in earthworm distribution and no significant correlation was detected between MAT and earthworm biomass or density. Though MAT is not correlated with earthworm density, MAT and MAP are correlated, so temperature may contribute to the relationship between MAP and earthworm density. The interaction between temperature and moisture could result in higher earthworm populations in some lower precipitation fields due to higher reproductive rates resulting from higher soil temperatures (Wever et al., 2001). No measured soil properties significantly correlated with earthworm biomass, density or climate variables ($p > 0.05$). Earthworm populations may be correlated to soil properties within an AEC, however, our ability to detect such relationships is reduced by the relatively low number of observations with in AECs (n between 10 and 15 for each AEC) may preclude detecting these effects.

Conclusion

This survey of IPNW crop fields for earthworms found a very low species diversity, with *A. trapezoides* the dominant species and *L. terrestris* found at only two sites. The ability to detect *L. terrestris* and other species that may be present at low abundances is limited by the sampling techniques available. Additionally, only adult earthworms could be identified to species. Therefore, sampling limited to one date each year may miss species with adults present earlier or later in the year. *Aporrectodea trapezoides* is a peregrine species found worldwide from Australia and New Zealand to Europe, North America, Algeria and Egypt (Fernandez et al., 2011). This species readily adapts to a wide range of conditions and it is not unusual for it to be the sole species present at a site (Fernandez et al., 2011). Despite its adaptability and ability to weather seasonal periods of inhospitable conditions, a threshold in

MAP between 330 and 370 MAP appears to limit the distribution of *A. trapezoides* in IPNW crop fields. Earthworms were found in all annual cropping fields and transition fields, but only found in 13% of crop-fallow fields where water is most limiting for crop production. When only sites with earthworms present are considered, an inverse effect of precipitation on earthworm population size is seen with a significant negative correlation for both the previous year's precipitation and MAP with earthworm biomass. This correlation may be an artifact of the sampling schedule in which lower MAP sites were generally sampled earlier in the season when soil temperature and moisture might be expected to be more favorable. Also, the AEC system provides an indication of how climatic factors such as MAP translate into soil water availability throughout the season. A higher MAP does not necessarily indicate greater soil water and the most water-limited AEC, crop-fallow had the lowest average densities.

Mean biomass numbers in this study were well above the 30 g m^{-2} cited for a significant impact on crop yield in tropical systems. The threshold, however, was reported for tropical systems, very different from the conditions found within the IPNW. The implications of earthworm density for crop yields in the IPNW are unknown and require further study. It is also interesting to point out the data reported in this paper may be useful in modeling the movement of *A. trapezoides* in the IPNW under expected, modeled climatic conditions.

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Tables

Table 2.1: Mean annual precipitation (MAP), spring precipitation (SPPT), winter precipitation (WPPT), and mean annual temperature (MAT) by agroecological class (AEC) for all sites and percent of sites with in each AEC with earthworms present (EW). MAP and MAT are calculated based on data from 1980-2010. SPPT includes the months of Mar-Jun and WPPT is based on Sept-Feb for the years 2011-2013. AEC effect significance from ANOVA.

AEC	n	MAP[†]	SPPT	WPPT	MAT	EW
		---------------mm---------------			---°C---	%
Annual	11	565^{a}	259^{a}	302^{a}	48.2	100^{a}
Transition	10	459^{b}	227^{a}	226^{b}	48.7	100^{a}
Crop-Fallow	15	313^{c}	145^{b}	175^{c}	48.2	13.3^{b}
AEC effect		$p<0.0001$	$p<0.0001$	$p<0.0001$	NS	$p<0.0001$

[†] Means within columns assigned different letters are significant ($p<0.05$)

Table 2.2: Mean values for pH, soil organic matter (SOM), sand, silt and clay particle sizes by AEC. AEC effect significance from ANOVA.

AEC	n	pH	SOM	Sand	Silt	Clay
			---------------------------------%---------------------------------			
Annual	11	5.65	4.0	31.5	52.1	16.4
Transition	10	6.21	4.6	30.9	50.3	18.8
Crop-Fallow	15	6.00	3.1	37.4	48.7	13.9
AEC effect		NS	NS	NS	NS	NS

[†] Means within columns assigned different letters are significant ($p<0.05$)

Table 2.3: Spearman correlation coefficients for earthworm biomass and density with the previous spring's precipitation (Spring ppt, March-June), the previous winter's precipitation (Winter ppt, Sept-Feb), mean annual precipitation (MAP, 1980-2010) and mean annual temperature (MAT, 1980-2010)

	Density	MAP	Fall ppt	Spring ppt	MAT
Biomass	0.9403^{***}	-0.4390^{*}	-0.7377^{***}	-0.6779^{**}	0.3143
Density		-0.39481	-0.6792^{**}	-0.6091^{**}	0.2377
MAP			0.4247	0.7221^{***}	-0.4221

*=p<0.05, **=p<0.01, ***p<0.001;

Figures

Figure 2.1: Map of study area, including agroecological classes (AECs) and sampling site locations.

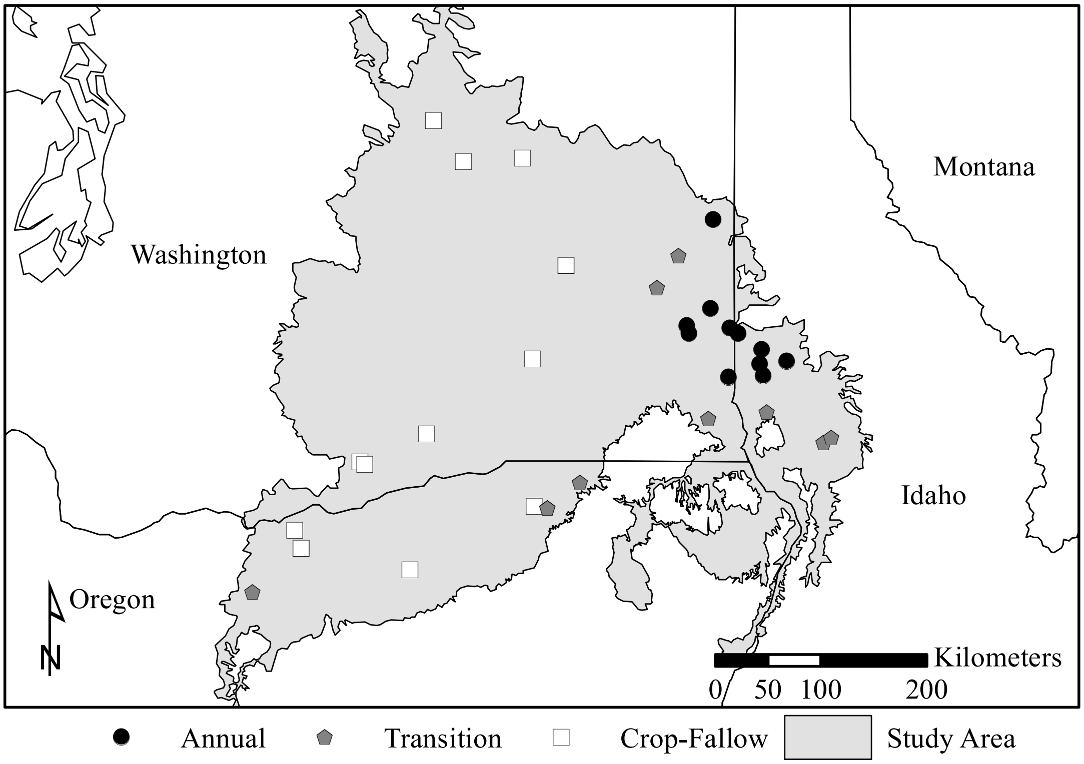


Figure 2.2: Mean earthworm biomass and density by agroecological class (AEC) for all sites with earthworms. Bars indicated standard error.

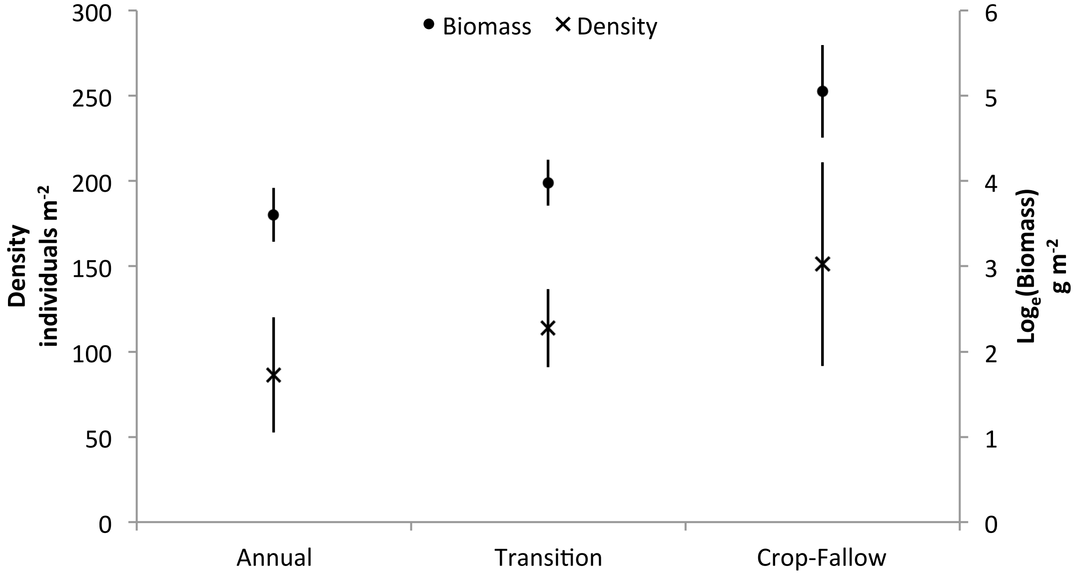


Figure 2.3: Mean earthworm biomass and density by year for all sites with earthworms. Bars indicated standard error.

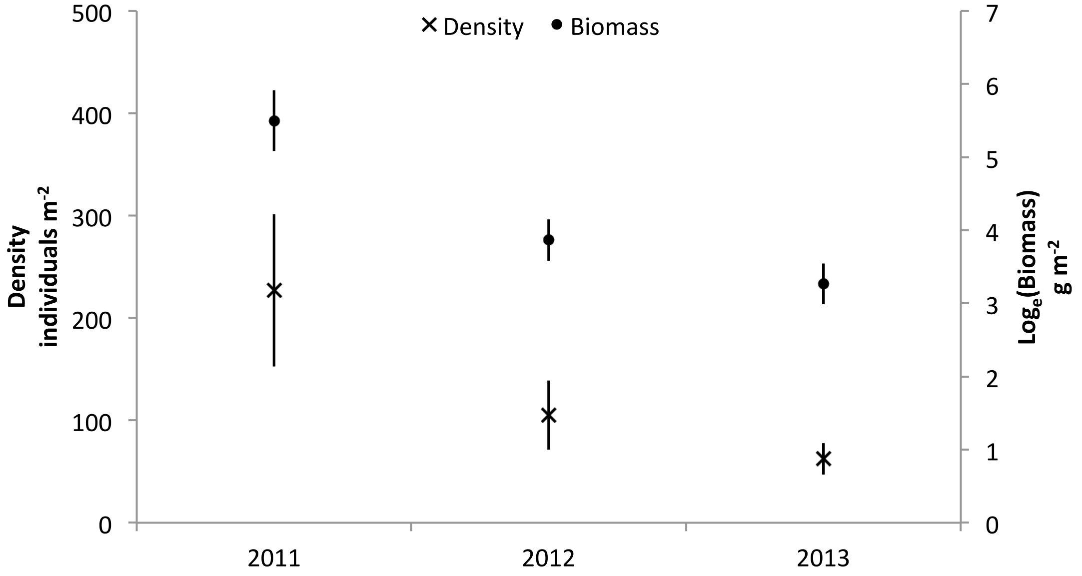
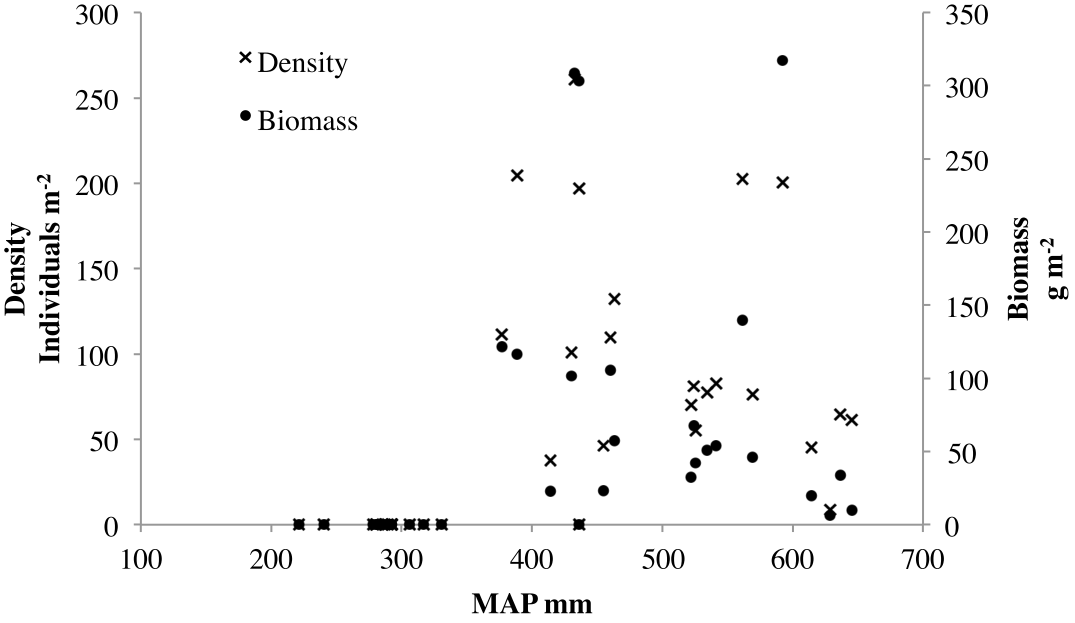


Figure 2.4: Mean earthworm biomass and density for all sites as a function of mean annual precipitation.



Chapter 3: Seasonal Variations in Exotic Earthworm Populations in Palouse Wheat Fields

Abstract

Exotic earthworms are found throughout the high yielding, dryland wheat-producing region of eastern Washington and northern Idaho known as the Palouse. Through their manipulation of soil physical and chemical properties, earthworms have the potential to increase nutrient mineralization and benefit crop production. However, this effect is dependent on numerous factors including earthworm density. Earthworms have only begun to receive more serious attention on the Palouse in recent years and only rough estimates of earthworm density and seasonal activity exist. Six Palouse wheat fields were monitored over the course of 14 months in 2014 and 2015 to describe seasonal variations in earthworm density and active periods. Earthworm community density, age structure and diversity, soil moisture and soil temperature were monitored over 14 months in 2014 and 2015. Only exotic species were collected and the endogeic species *Aporrectodea trapezoides* was the predominant species collected at all sites (87% of all adults identified). *Allolobophora chlorotica, Lumbricus terrestris* and *Aporrectodea tuberculata* were also collected at lower frequencies (1.8-6.4% of adults). In 2015, earthworms were active for 121 days with average densities of 14 to 75 m^{-2} during this period. Population transition to aestivation began in mid June of both years and appears to be driven by an interaction between soil moisture soil temperature. A short active period and average densities at most sites less than 100 individuals per m^2 suggest the impact of *A. trapezoides* may be limited in conventional crop fields of this region. However, in organic or other limited input systems earthworms have greater potential to make a measurable contribution to nutrient mineralization.

Introduction

The Palouse region of eastern Washington state and northern Idaho is known for its deep loess soils and high wheat yields (Black et al., 1998). Originally a region of loess dunes covered by bunchgrass prairie (Black et al., 1998), the Palouse is home to at least one species of native earthworm, *Driloleirus americanus* (Smith), the Giant Palouse Earthworm (James, 2000; Sanchez-de Leon and Johnson-Maynard, 2009; Xu et al., 2013). *Driloleirus americanus* has been found in remnants of native prairie habitat but never in agricultural fields of this region (James, 2000). The conversion to agriculture from native prairie in this region has been accomplished by intensive cultivation and accompanied by high erosion rates (Black et al., 1998). In this high-disturbance agroecosystem, exotic earthworm species now dominate the landscape (Fauci and Bezdicek, 2002; James, 2000; Sanchez-de Leon and Johnson-Maynard, 2009; Chapter 2, this thesis). Previous surveys of the Palouse region have found ten exotic Lumbricidae species within agricultural fields, waterways, old home sites and other habitats (Fauci and Bezdicek, 2002). Within crop fields the endogeic *Aporrectodea trapezoides* (Dugés) is the dominant species collected by far with only occasional specimens of the endogeic species, *Aporrectodea tuberculata* (Eisen) and *Aporrectodea turgida* (Eisen) and the anecic species, *Lumbricus terrestris* (L.) (Fauci and Bezdicek, 2002). Endogeic is a term used to categorize earthworms that create impermanent horizontal burrows within the soil, consuming soil organic matter (Bouché, 1977). Anecic species create permanent vertical burrows and consume organic residue from the surface of the soil (Bouché, 1977). A third category, epigeic, do not burrow within the soil at all but rather inhabit the interface between the O horizon and mineral soil (Bouché, 1977).

Despite the negative stigma frequently associated with exotic species, *A. trapezoides* has the potential to have a beneficial effect on crop production on the Palouse. Earthworm activity has been linked to the improvement of many soil physical and chemical properties (Baker et al., 2006; Bertrand et al., 2015; Carpenter et al., 2008; Costello and Lamberti, 2009; Coulis et al., 2014; Jones et al., 2006; Lavelle et al., 1997; Syers and Springett, 1984) and the bulk of their impact on crop production is thought to result from increased nutrient mineralization rates (Blair et al., 1997; van Groenigen et al., 2014). *Aporrectodea trapezoides* in particular has been found to significantly increase wheat yield (Baker et al., 1997; Doube et al., 1997), and increase mineralization of nitrogen from soil organic matter (Postma-Blaauw et al., 2006; Whalen et al., 2000) in greenhouse studies. Although these greenhouse studies generally use higher densities of *A. trapezoides* (Baker et al., 1997; Doube et al., 1997) than reported in the Palouse region (Johnson-Maynard et al., 2007; Umiker et al., 2009), in general earthworm effects on crop production can be significant even at low densities (van Groenigen et al., 2014). Many factors limit the application of greenhouse data to field conditions. In the case of earthworm effects on crop growth the measured effect of earthworms is generally higher when disturbed soil is used (van Groenigen et al., 2014), and therefore may overestimate the effect of earthworms relative to field conditions. On the other hand, the effect of earthworms on nitrogen mineralization and N_2O emissions are known to increase with experimental period (Lubbers et al., 2013). Therefore, the relatively short length of greenhouse studies may underestimate the potential impact of earthworms.

Meta-analyses of the effect of earthworms on crop production across all species and crops have estimated increases in yield of 25-35% when earthworms are present (Brown et al., 1999; van Groenigen et al., 2014). Earthworm effects are significantly greater when earthworm densities are greater than 400 individuals m^{-2} compared to less than 100 individuals m^{-2} but earthworm effects were significant at all densities (van Groenigen et al., 2014). Earthworm densities for *A. trapezoides* have been reported between 20 to 110 earthworms per m^2 in the Palouse (Johnson-Maynard et al., 2007; Umiker et al., 2009). However, these earthworm densities reflect single sampling dates in early spring, when the cool wet soil conditions are thought to be optimal for earthworm activity (Johnson-Maynard et al., 2007; Umiker et al., 2009). How these populations change across their active period, the length of their active period and the average seasonal density of this species are not known.

The Mediterranean climate of the Palouse region produces dramatic seasonal fluctuations in soil conditions from saturated soils in the winter, that can freeze to depths of 40 cm, to hot, dry soils in the summer (Papendick, 1996; Schillinger and Papendick, 2008). Highly sensitive to soil moisture and temperature, earthworm population density and activity are in turn expected to vary temporally (Eriksen-Hamel and Whalen, 2006; Perreault and Whalen, 2006; Presley et al., 1996; Richardson et al., 2009; Wever et al., 2001). Both the hot dry soils of the summer and cold saturated soils during winter are expected to limit the activity and density of earthworm populations in this region.

Aporrectodea trapezoides is a flexible species and is potentially able to survive inhospitable conditions using a number of strategies. First, earthworms can migrate down in the soil profile where soil moisture and temperature may be more favorable (Curry and Schmidt, 2007; Lee, 1985). However, the presence of restrictive layers too dense to burrow through and lack of food in the form of soil organic matter limit the migration depth of endogeic earthworms (Curry and Schmidt, 2007; Lee, 1985). In these cases the earthworm either begins aestivation or dies (Curry and Schmidt, 2007; Lee, 1985). Aestivation is a form of diapause practiced to varying degrees by a number of earthworm species (Juan et al., 2000; Lee, 1985). During aestivation the earthworm forms a spherical chamber where it remains tightly coiled with reduced metabolic rates until soil conditions are once again favorable (Juan et al., 2000; Lee, 1985). Aestivation is often associated with moisture stress but can occur at otherwise adequate soil moisture levels when heat and water stress are combined (Wever et al., 2001). How long earthworms can survive aestivation is not well known. One laboratory study with *Aporrectodea calignosa* (closely related to *A. trapezoides*), found that after 3 weeks of drought 72% of earthworms were aestivating and the overall mortality rate was 14% (McDaniel et al., 2013). Although aestivation allows individuals to survive periods of inhospitable conditions earthworms are not modifying the soil during these periods.

Cocoon production is another ecological strategy allowing earthworm populations to survive periods of inhospitable conditions (Edwards and Bohlen, 1996). *Aporrectodea trapezoides* reproduces sexually and parthenogenically through the production of cocoons, small tough cases in which hatchlings develop (Edwards and Bohlen, 1996; Lee, 1985). Cocoons are resistant to both desiccation and cold, and delay hatching until soil conditions are more favorable (Edwards and Bohlen, 1996). In the lab, individual *A. trapezoides* produce as many as 105 cocoons a year, which take 42 days to develop (Fernández et al., 2010). *Aporrectodea trapezoides* will reproduce parthenogenically even when other individuals are present (Fernández et al., 2010).

The importance of soil biology and soil quality for crop production and sustainability has gained increasing attention in recent decades and earthworms and other soil invertebrates have long been used as indicators of soil quality (Doran and Zeiss, 2000; Glanz, 1995;

Pankhurst et al., 1995). Earthworms are well known for their potential positive impacts on numerous soil properties related to soil quality (Jones et al., 2006; Lavelle, 1997; Lavelle et al., 1997; Lee, 1985; Syers and Springett, 1984) and are expected to play a key role cycling nutrients in organic systems (van Groenigen et al., 2014). A better understanding of how earthworm populations currently function in agricultural regions such as the Palouse is a key step to understanding the role they may play in fostering resiliency now and in the future. In this study earthworm populations at six sites across the Palouse region were surveyed over the course of 14 months. The density, activity and age structure of the earthworm populations, and soil moisture and temperature were monitored to obtain a better idea of temporal variations in earthworm activity in this region and their relation to soil conditions.

Methods

Site Characterization

Six sites were selected to represent the range of mean annual precipitation and agronomic practices found across the annual crop-producing region of the Palouse. Modeled mean annual precipitation (MAP) and temperature (MAT) data were obtained from the West Wide Drought Tracker Service (Table 3.1). Mean annual temperature and precipitation data were generated using interpolation by the Oregon State University PRISM Climate group for the years 1981 to 2010 (PRISM, 2014). Soils at all sites are mapped as either Haploxerolls or Argixerolls (Soil Survey Staff, 2015). Sampling areas were located on northeast to northwest facing slopes and at low mid-slope positions. Soil pH was measured using a 1:1 (soil:water) dilution (NRCS, 1992) and total organic matter was measured using the loss on ignition method (NRCS, 1996). Bulk density samples were collected after harvest and before fall cultivation in 2014 using a 20 cm x 6 cm diameter core (Table 3.1).

During the first growing season, winter wheat was planted at all sites (Table 3.2) and in spring of 2015 canola, wheat and barley were planted with one site remaining fallow. Tillage at the study sites included a range of intensities. Sites 1, 2 and 6 were all no-till, direct-seed fields with no cultivation. Sites 4 and 5 had similar disturbance levels with chisel plowing used in the fall after harvest and harrowing or shallow chisel used in the spring to prepare the seed bed. Site 3 had the widest range of cultivation intensity. At site 3 in years where peas were planted no or minimum tillage is used. Other years a chisel was used and a plow was used ever second or third year for less than half of the acreage. In the fall of 2013 and 2014 a moldboard plow was used at site 3 and buried residue at 20 cm. A harrow was also used in the spring to prepare the seed bed for planting.

Soil Moisture and Temperature

Soil temperature at 20 cm from two replicate Decagon RT-1 temperature probes was recorded using Decagon EM50 data loggers (Decagon, Pullman, WA) were installed at each site and. Data loggers were removed in July of 2014 and were not replaced until November of that year to allow producers to cultivate fields before winter. Soil moisture was measured at the time of earthworm collection, horizontally at 20 cm within each of three earthworm pits per site, using a Campbell HydroSense II portable moisture sensor (Campbell Sci., Logan, UT) and averaged across all three pits for each date. Heterogeneity in soil moisture was similar at all sites with high and low soil moisture values at each site for a single date varying between 0.3 and 16.9% (absolute). Gravimetric moisture content was collected at all sites from each pit three times over the project (9 data pairs total) and converted to volumetric moisture content using measured bulk density values. To account for differences in soil physical properties, best-fit linear regression calibration was used as needed for Individual sites to bring values within 5% (absolute) of the measured gravimetric values.

Earthworm Sampling

Earthworm collection occurred within a 15-m radius of the data logger and soil temperature probe location. Earthworms and cocoons were sieved when soil coil conditions permitted and hand sorted when necessitated by high soil moisture. Hand sorting and sieving are generally considered the most accurate method after wet sieving for sampling all ecological earthworm groups (Bartlett, et al., 2010; Lee, 1985). Epigeic species remain on the soil surface and therefore are can be collected in very shallow pits, or by removing the residue layer. Polyhumic endogeic earthworms remain in the upper, organic rich, top soil layers and the majority of *A. trapezoides* in this region have been found in the top 20-30 cm of the soil (Umiker, et al., 2009). However, anecic burrows can extend below the depth practical for sampling using the hand sorting method and are likely to be underrepresented relative to shallower burrowing endogeic and epigeic species (Chan, 2004). On each sampling date, at each site, three pits (25x25x30 cm) were randomly located, each within one of three zones: up slope, even with, and down slope of the data logger. Subsequent pits were located at least 1 m away from disturbed soil. Cocoons were counted at the site and returned to the pits. The presence/absence of aestivating individuals was recorded at the time of sampling. Excavation combined with hand sorting and sieving frequently destroys aestivation chambers and results in the uncoiling of aestivating worms. Therefore, aestivation was determined either by excavation of intact aestivation chambers containing coiled earthworms (usually also unresponsive and dehydrated) or by excavation of similarly dehydrated and unresponsive individuals. Earthworm collection began 10 April 2014 and continued through 25 June 2015. Earthworms were collected on 19 dates during this 14-month period.

In the laboratory, live biomass was determined after gut contents were cleared by incubating earthworms at 15°C for 48 hours on filter paper saturated with a 1:8 Ringer's solution (Dalby et al., 1996). Earthworms were separated into three age classes, adult, sub-adult and juvenile, then counted and weighed separately. Individuals with a fully formed clitellum were classified as adult, individuals with some development of the tubercula puberatis or genital tumescence but without a clitellum were classified as sub adults, and individuals with no development of tubercula puberatis or genital tumescence were classified as juveniles. Adult earthworms were euthanized and preserved in formalin then identified to species using Schwert's key to Lumbricidae (Schwert, 1990).

Statistical Analysis

Average biomass and densities for each site were calculated only from dates during which all earthworms were active and were weighted by days between sampling dates. Cumulative soil degree-days (CSDD) were employed as a more biologically relevant measure of temperature for ectotherms than discreet daily soil temperature data (Atkinson, 1994; van Straalen, 1983). Cumulative soil degree-days were calculated using equation 1. A base temperature (T_b) of 5°C was used and CSDD accumulation began on 15 April in 2014 and 2015 to allow comparison between years. Data to calculate CSDD beginning 1 January were available for 2015 only.

$$\text{Equation 1: } \left(CSDD = \frac{T_{max} - T_{min}}{2} - 5\right)$$

The data were analyzed using SAS software, Version 9.4 of the SAS System for Windows, SAS Institute Inc. (SAS, 2013). Spearman rank-order correlation was used to compare soil moisture and cumulative soil degree-days with earthworm biomass and density and cocoon density and included only data from dates when earthworms were present.

Results and Discussion

Soil Moisture and Cumulative Soil Degree-days

Soil moisture followed a similar pattern across all sites decreasing sharply from April to June of 2014 (Figure 3.1). In the fall of 2014, soil moisture did not recover until November and then remained relatively stable until June of 2015. Nearby weather station measurements (<8 km) were available for sites 6 and 5. At site 6, 480 mm of rainfall was recorded between October 1, 2014 and July 2015, while that site only received 295 mm of rainfall for the same period the preceding year. Rainfall was also higher October 2014 to July 2015 at site 5, which received 648 mm in 2015 and 518 mm in 2014. While the other sites were not located near weather stations, the general pattern of higher rainfall in 2015 as compared to 2014 was consistent for the entire region. In 2014, the fields were planted with winter wheat, while in 2015 fields were either planted with spring crops or left fallow. The later germination and development of spring crops, relative to fall planted winter wheat, may also have contributed to the delayed decline in soil moisture in 2015.

Cumulative soil degree-days also had similar seasonal patterns across sites with less variation between sites than soil moisture (Figure 3.2). Cumulative soil degree-days increased more rapidly in 2015 when record high temperatures were recorded across the region; average soil temperature across sites was 2 degrees higher on 15 April 2015 than 15 April 2014.

Species

Four species were collected over the course of the study: three endogeic species, *Aporrectodea trapezoides, Aporrectodea tuberculata* and *Allolobophora chlorotica,* and one anecic species, *Lumbricus terrestris*. Additionally, two adults were collected at site 3 which could not be definitively identified to species and were not included in calculations of species abundance. One was identified as belonging to the genus *Aporrectodea* and the other as belonging to the *Aporrectodea calignosa* complex. This complex includes two species previous found in crop fields of the Palouse *A. trapezoides, A. tuberculata,* and three additional species *A. calignosa, A. nocturna* and *A. longa* (Pérez-Losada et al., 2009). Species within this complex are often difficult to differentiate and have variable morphology (Pérez-Losada et al., 2009). Similar to previous surveys of agricultural fields *Aporrectodea trapezoides* was the dominant species, collected at all six sites and making up 87% of the individuals identified (Table 3.3). *Aporrectodea tuberculata* (6.4% of adults) and *Lumbricus terrestris* (1.8% of adults) were also found in previous surveys, while *Allolobophora chlorotica* (4.6% of adults) had been found in natural areas adjacent to agricultural fields but not within agricultural fields themselves (Fauci and Bezdicek, 2002). Site 4 had the highest diversity, with all four species present, including *A. chlorotica* and *L. terrestris*, which were only collected at that site. At sites 2 and 6 only *A. trapezoides* was collected.

Adult *A. trapezoides* and *A. tuberculata* were collected under a wide range of soil moisture conditions, 8-39% volumetric (Table 3.3), while *L. terrestris* was only collected from soils with moisture contents greater than 35%. The hand-sorting/sieving method used in this study may underestimate the presence of anecic earthworms (Callaham and Hendrix, 1997). So it is possible that *L. terrestris* is present at other sites and surely present throughout the year where they are known to be present, but was not collected as a result of sampling limitations. Differences in maturation rates, and winter/summer survival rates among species may also affect the measured diversity of earthworm species by effecting the period during which adults are available for identification. Information on development times for *A. chlorotica* (56-84 days) (Lowe and Butt, 2005), *L. terrestris* (90-214 days) (Lowe and Butt, 2005) and *A. trapezoides* (142-165 days) (Fernández et al., 2010) in laboratory conditions suggest that the first appearance of adults of each species would not be expected at the same time and multiple sampling dates is likely to be necessary to ensure all adults are represented.

Earthworm Biomass and Density

Average earthworm biomass during the active period of the year ranged from 6.7 g m^{-2} at site 2 in 2014 to 61.5 g m^{-2} at site 3 in 2014 (Table 3.4). Site 2 tended to have the lowest earthworm density and biomass values. This site also has the lowest MAP (Table 3.1). In at least one of the two years, all sites but 2 had earthworm biomass values over the 30 g m^{-2} necessary for agriculturally significant increases in grain yields in tropical systems (Brown et al., 1999). Average earthworm densities generally less than 100 earthworms per m^2; a level at which earthworm effects vary greatly from less than 5% to as high as 30% (van Groenigen et al., 2014). Highest seasonal average earthworm density for all sites and years was 149 individuals m^{-2} at site 3 in 2014. Another study, in Western Australia, also monitored density of *A. trapezoides* across their wet season (May-October) and reported that *A. trapezoides* populations in the top 10 cm fluctuated between 58 and 107 individuals m^{-2} (McCredie et al., 1992). These densities fall with in the range recorded in this study, where the highest density at a single sampling date (average for all 3 pits) was 320 individuals m^{-2} (site 3, April 9th, 2014).

Earthworm biomass and density were highly variable between sites but followed similar patterns. Earthworm biomass and density (Figure 3.3a and 3.3b) were generally highest during the spring months, diminishing gradually as the soil warmed and dried out, then precipitously after aestivation began, suggesting high levels of mortality. Earthworm densities and biomass decreased more quickly during 2014 compared to 2015, corresponding to a steeper decrease in soil moisture in 2014 than in 2015. When sampled during the winter of 2014-2015 earthworms were active as early mid February and the highest densities in 2015 were collected at this early sampling date while soil moisture was high and soil temperatures at the 20-cm depth were close to 5° C.

Across all sites and dates soil moisture had a significant positive correlation with biomass and density ($p<0.001$) while CSDD correlated negatively with earthworm biomass ($p<0.001$) and earthworm density ($p<0.01$) (Table 3.5). Soil moisture and CSDD are also negatively correlated with each other ($p<0.001$) making it difficult to determine the relative importance of temperature (in this case CSDD) and soil moisture as potential drivers of earthworm populations. Indeed, the literature suggests that these factors should not be considered independently since earthworms' response to temperature varies with water availability (Perreault and Whalen, 2006; Presley et al., 1996; Wever et al., 2001).

Population Structure

Similar to earthworm density and biomass, age structure was highly variable among sites but with a relatively consistent seasonal patterns at all sites. In 2014 adult earthworms, on average, constituted less than 5% of the population at the beginning of the season (Figure 3.4). This proportion gradually increased until mid May, peaking at an average of 32%. Densities of adults dropped to zero about one month after aestivation began and remained low until the following spring. The reappearance of adults in October of 2014 represents a single adult collected at that date. Some species of earthworms are known to reabsorb the clitellum and gonads during aestivation (Juan et al., 2000), which, along with mortality, likely accounts for the disappearance of adult earthworms during periods of inactivity. Adult earthworms may also have had greater ability to burrow below the 30 cm sampling depth. In 2015, the proportion of adults at the first sampling date (average of 18%) was higher than in 2014 and remained higher for most of the season. Adults accounted for roughly half of the population between April 16th and June 2nd in 2015. This sustained period of adult activity parallels the higher soil moisture during this period as compared to 2014. In 2015, adults maintained 18-20% of the population during the first two weeks of aestivation. Juveniles were also more prevalent in the early season and adult numbers peaked late in the season (late August-early Sept) in the Western Australian study (McCredie et al., 1992). In the Australian study (McCredie et al., 1992) and another in England (Evans and Guild, 1948) the active season for earthworms began in the early fall and adults were not present until two-four weeks after the first fall rains following the dry season. In this study adults were present on the first sampling date, as early as February in 2015. The reappearance of adults early in the season suggests that some sub adults and juveniles survive the winter and may begin to develop into adults while soil temperatures are still quite low. Adults aestivating below 30 cm may also account for adult presence early in the season.

Relatively few cocoons were collected throughout 2014, and in 2015 the highest number of cocoons were collected in early June, roughly 6 weeks after the highest proportion of adult earthworms were collected (Figure 3.4). A similar delay was observed with *A. trapezoides* in Australia where cocoons were first collected 6.5 weeks after the first adults were collected

(McCredie et al., 1992). Cocoon numbers did not show a decline with aestivation in 2014, most likely as a result of inhospitable conditions delaying hatching (Edwards and Bohlen, 1996). In 2015, there was a general decline in cocoons within the month of June, however, the data showed considerable variability. No significant correlation was found between either soil moisture or CSDD, and cocoon density. Cocoon numbers may have been underestimated in this study, as the small cocoons can be difficult to identify especially during wet periods when hand sorting is necessary and cocoons retain a coating of mud (Bartlett et al., 2010).

Aestivation

Aporrectodea trapezoides was the only species identified after the beginning of aestivation. In 2014, the first aestivating earthworm was collected on 4/5 June for sites 1, 3 and 4 and on 17/18 June at sites 2, 5 and 6. In 2015, aestivating earthworms were found at all sites on 17/18 June, except site 6 where the first was found 25 June. Between 17 June and 19 February there were 121 days in which all earthworms were active and 244 days during which some or all earthworms were aestivating from 17 June 2014 to 19 February 2015.

Cumulative soil degree-days at the onset of aestivation for all sites varied between 360 and 761. The onset of aestivation occurred later in 2015 at all sites, from 34 CSDD later at site 5 to 400 CSDD later at site 1 (Figure 3.5). Soil moisture was higher at the onset of aestivation for all sites in 2015 compared to 2014 and this interaction may account for some of the difference in CSDD between years, as higher soil moisture allows earthworms to tolerate greater heat stress (Wever et al., 2001). It was possible to calculate CSDD starting 1 January, for 2015 only. These numbers were 37 to 223 degree-days higher among sites than CSDD starting 15 April and place the start of aestivation between 595 and 918 CSDD (Figure 3.5). The two-week gap between sampling dates may also account for some of the disparity between years and sites as aestivation could have begun as much as two weeks before it was first observed in most cases. Both aestivating and active earthworms were collected when CSDD were between 361 and 496 suggesting a potential threshold that varies with moisture across that range of CSDD (Figure 3.6).

Soil Properties and Agronomic Management

Soil pH was consistently acidic across sites ranging from 4.7 to 5.9 (Table 3.1), which falls within the range earthworms are known to tolerate (Curry and Schmidt, 2007) and no relationship between soil pH and mean earthworm density or biomass was observed. Bulk density for the 0-to 20-cm depth for the sites ranges between 1.14 and 1.24 g cm^{-3} except site 3, which has an unusually low bulk density of 0.89 g cm^{-3} (Table 3.1). The highest earthworm densities were found in the low-density soils of site 3 but beyond that no clear effect of soil bulk density on earthworm density was found. Soil organic matter for the sites fell between 3.3 and 4.7% for all sites except again site 3 where the soil organic matter was 6.1% (Table 3.1).

Numerous studies have investigated the effect of tillage on earthworm population density and diversity and have reported a wide range of positive, negative and neutral effects (Bertrand et al., 2015; Chan, 2001; van Capelle et al., 2012). Although a variety of tillage intensities are represented in this study, its design does not allow conclusions to be drawn regarding the effect of tillage intensity on earthworm populations. When all sites and years are considered there is no apparent relationship between tillage intensity and earthworm density. Previous work in the Palouse region also reported mixed findings with no statistical difference between earthworm density across conservation and no-till sites in one study (Umiker et al.,

2009) but an increase in density three years after conversion to no-till in another (Johnson-Maynard et al., 2007).

Conclusion

This study found similar species diversity compared to previous studies with *A. trapezoides* being the dominant species found within agricultural sites in the Palouse region. The ubiquity of this species was not surprising given its predominance in previous earthworm surveys and studies in the region (Fauci and Bezdicek, 2002; Johnson-Maynard et al., 2007; Sanchez-de Leon and Johnson-Maynard, 2009; Xu et al., 2013). Additionally, *A. trapezoides* is well known for its adaptability and is found worldwide in a variety of climates, frequently as the only species present at a site (Fernandez et al., 2011). *Allolobophora chlorotica* was found at only one site and had not been previously collected from agricultural sites in this region, though it had been collected from natural habitats and home sites bordering agricultural fields (Fauci and Bezdicek, 2002). *Allolobophora chlorotica* has also been found throughout the northern and central Western US including areas along the Snake River in southern Idaho and forested, higher organic matter environments along the Idaho-Montana boarder (Fauci and Bezdicek, 2002; Fender, 1985; Gates, 1967). *Aporrectodea tuberculata* had not been collected from Palouse agricultural fields since 2002 (Fauci and Bezdicek, 2002). The species *A. chlorotica* and *L. terrestris* were collected in low numbers at only one site on only a quarter of the sampling dates overall. The relative rarity of these species suggests that more intensive sampling both across the landscape and over time is necessary to obtain a complete catalogue of earthworm species (Bartlett et al., 2010). Differences among species in time to maturity and the window during which adults may be collected present significant constraints on the ability of researchers to obtain a complete picture of earthworm diversity. The limitations of available earthworm-sampling methods further constrain our ability to detect deep burrowing endogeic or anecic species, especially when present in low densities. New low disturbance techniques utilizing environmental DNA are promising for obtaining a more complete survey of earthworm diversity across a wide range of habits and warrant greater attention. Unfortunately, these techniques will likely have a limited capacity for determining earthworm densities.

Average active earthworm densities were much lower than those at which *A. trapezoides* has been shown to significantly impact wheat growth in greenhouse studies. Also, during the 14 months of this study the longest period of earthworm activity was only 121 days, roughly a third of the year. The length of these periods of activity and earthworm density can be expected to vary temporally and spatially as a result of climatic, topographic and soil heterogeneity. Additionally, the distribution of earthworms across the varied topography of the Palouse, the effects of temperature, moisture, and organic matter source on mineralization rates and the effects of management and crop rotation on *A. trapezoides* density are largely unknown. Despite the many uncertainties remaining regarding the effect of earthworms on Palouse agriculture, the positive effect of high densities of *A. trapezoides* on wheat growth in the greenhouse (Baker et al., 1997; Doube et al., 1997), and the significant positive effect of even low densities of earthworms in general (van Groenigen et al., 2014), together suggest that further investigation of the effect of *A. trapezoides* on crop production in the Palouse is warranted. High inputs of synthetic nitrogen in this region may also limit the potential impact of earthworms in conventional systems. However, for producers seeking to transition to organic or lower input systems, an understanding of controls on earthworm contributions to nutrient mineralization will be important to maximize their positive impacts.

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Tables

Table 3.1: Site Climate and Soil Characteristics: Aspect, mean annual precipitation (MAP), mean annual temperature (MAT), and soil classification. Also, bulk density, 1:1 pH and percent organic matter by loss on ignition (LOI) for 0-20 cm for each site.

Site	Aspect	MAP	MAT	Soil	Bulk Density	pH	LOI
		mm	*°C*		*g cm^{-3}*		*%*
1	WNW	480	9.6	Ultic Haploxerolls	1.21	5.08	3.3
2	NNE	470	9.9	Pachic Haploxerolls	1.14	4.90	3.7
3	NE	490	9.7	Cumulic Haploxerolls	0.89	5.90	6.1
4	NE	609	10.0	Typic Argixerolls/ Ultic Haploxerolls	1.25	4.67	4.7
5	NW	638	8.5	Typic Argixerolls	1.24	5.53	3.9
6	NNE	593	8.1	Ultic Haploxerolls	1.15	5.12	4.3

Table 3.2: Site Agronomic Management: 2014 and 2015 crop species and tillage for each site. S=Spring F =Fall

Site	2014 Crop	2015 Crop	Tillage
1	Winter Wheat	Spring Wheat	No Till: 40 years
2	Winter Wheat	Fallow	No Till: 20 years
3	Winter Wheat	Barley	F Plow or chisel S harrow
4	Winter Wheat	Spring Wheat	F Chisel S harrow
5	Winter Wheat	Canola	F Chisel S chisel and/or harrow
6	Winter Wheat	Canola	No Till: 15 years

Table 3.3: Species composition: the percent of adult earthworms at all sites identified as *Aporrectodea trapezoides, Allolobophora chlorotica, Aporrectodea tuberculata* and *Lumbricus terrestris,* the number of sites at which these species were present, the range of soil moistures (volumetric) at which they were found and the ecological classification for each.

	% of all individuals	**# of sites present**	**Soil moisture range**	**Ecological classification**
	(n=221)	(n=6)	% m^3/m^3	
A. trapezoides	87.2	6	8-39	Endogeic
A. chlorotica	4.6	1	23-37	Endogeic
A. tuberculata	6.4	4	14-35	Endogeic
L. terrestris	1.8	1	35-40	Anecic

Table 3.4: Average earthworm biomass and density for dates without aestivating earthworms for each site.

	Biomass		Density	
	2014	2015	2014	2015
	----------------g m^{-2}----------------		----------------m^{-2}----------------	
1	40.2	52.0	65.1	61.3
2	6.7	14.4	8.5	14.0
3	61.5	24.1	148.9	74.6
4	50.2	32.9	65.6	66.6
5	31.7	28.6	72.9	49.5
6	43.7	39.8	60.2	52.2

Table 3.5: Spearman correlation coefficients for earthworm biomass and density with soil moisture and cumulative soil degree-days (CSDD).

	Biomass	Density	CSDD
Soil Moisture	0.547^{***}	0.469^{***}	-0.427^{***}
CSDD	-0.559^{***}	-0.361^{**}	

**=p<0.01, ***p<0.001;

Figures

Figure 3.1: Volumetric moisture at 20 cm for each site and sampling date.

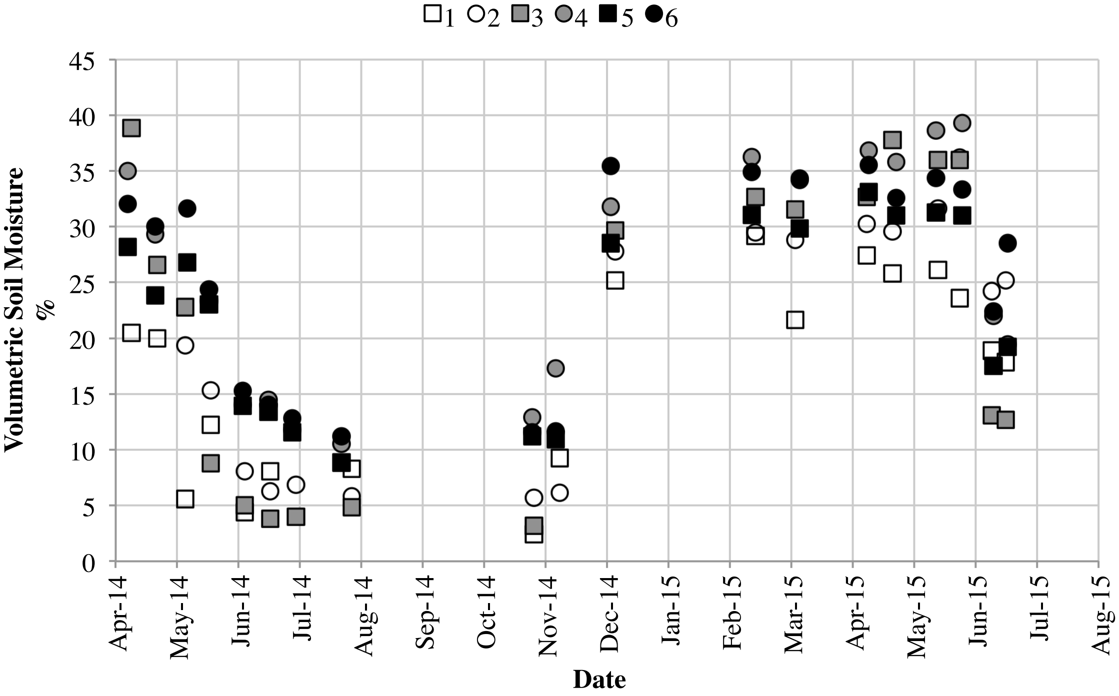

Figure 3.2: Cumulative soil degree-days (CSDD) from April 15th at 20 cm for each site and sampling date.

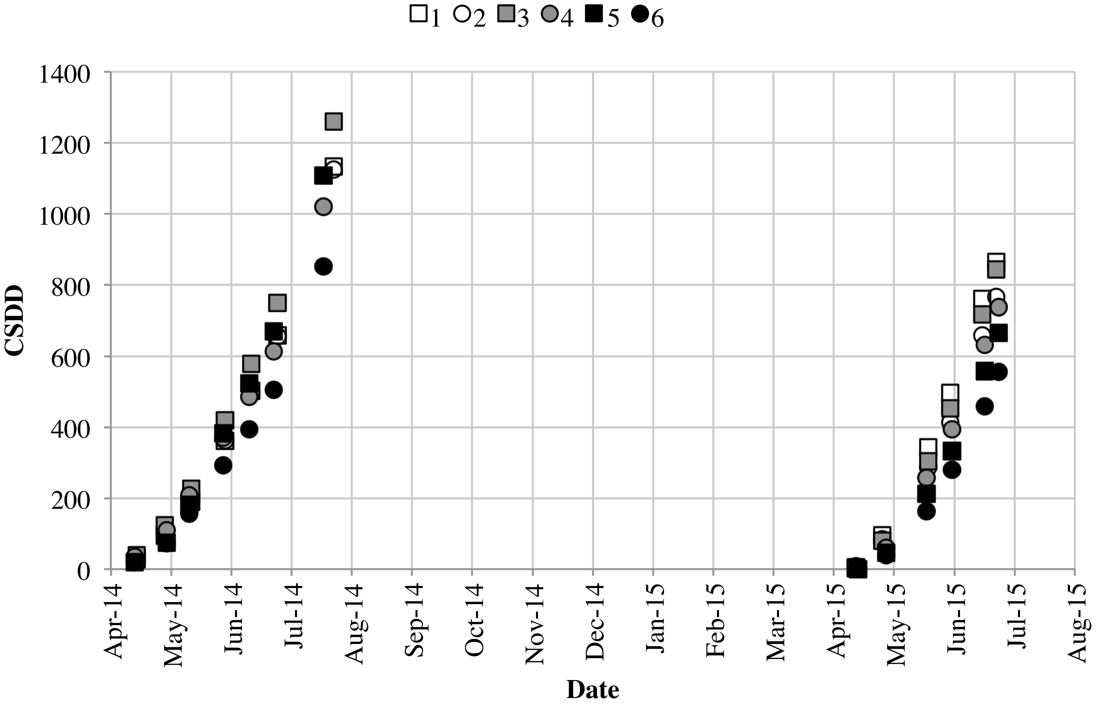

Figure 3.3: Boxplot of earthworm a) density (individual m^{-2}) and b) biomass (g m^{-2}) at all sites for each sampling date. Solid horizontal bars indicate periods where aestivating earthworms were present at all sites.

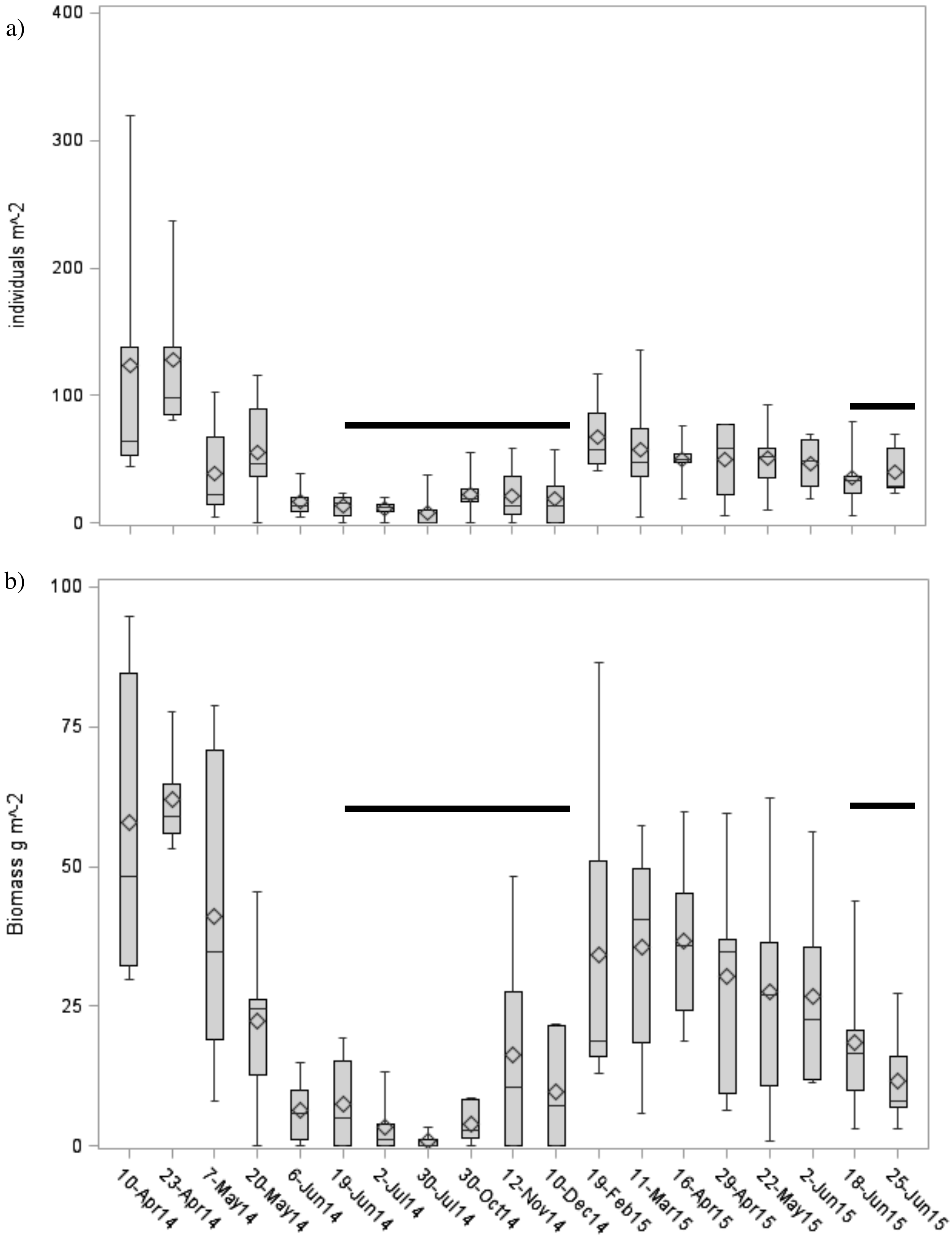

Figure 3.4: Proportion of earthworm population adult, sub adult and juvenile (by density), total density and cocoon density. Dates with black outlines indicate aestivating earthworms present at all sites.

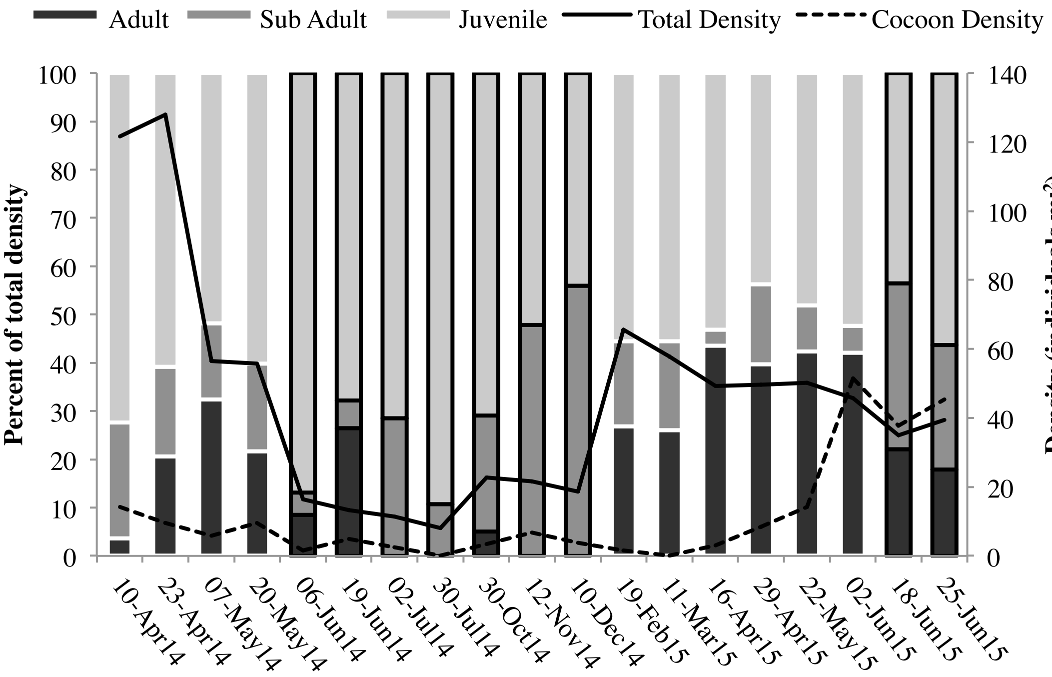

Figure 3.5: Cumulative soil degree-days (CSDD, T_b=5 °C, starting April 15th) and volumetric soil moisture at sampling date when earthworms began aestivating. Data labels in 2015 indicate CSDD beginning January 1st.

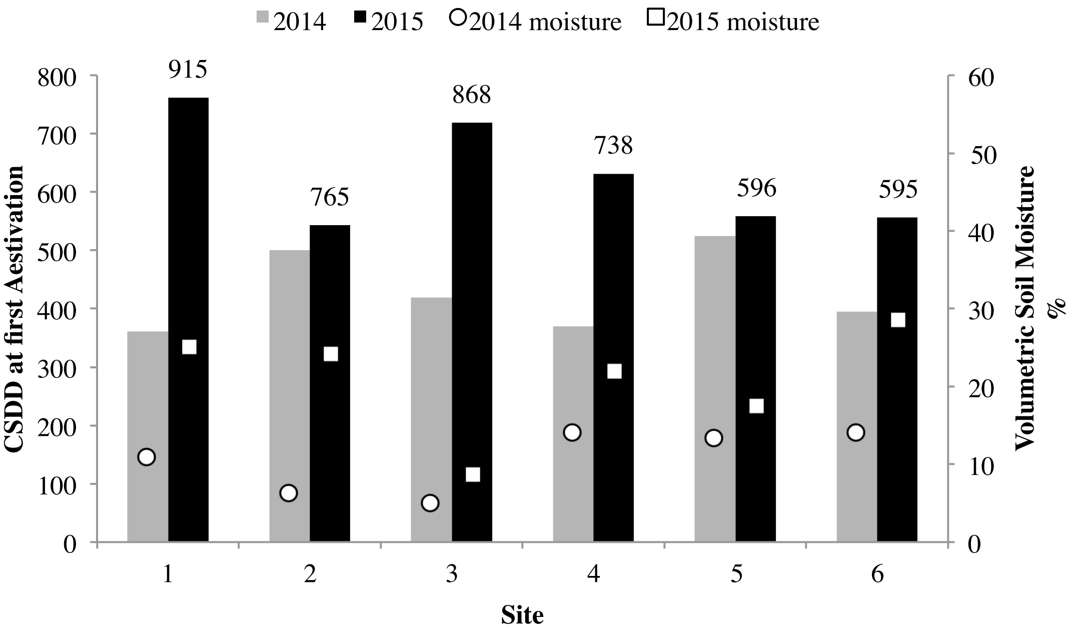

Figure 3.6: All sites cumulative soil degree-days (CSDD) by presence/absence of aestivating earthworms; data labels indicate the limits of the range with both active and aestivating individuals.